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<u>L11</u>	(allergen) same (hemagglutinin adj A)	1	<u>L11</u>
<u>L10</u>	L8 and (hemagglutinin adj A)	2	<u>L10</u>
<u>L9</u>	L8 and (hemagglutinin adj A)	2	<u>L9</u>
<u>L8</u>	L7 and (ragweed or pollen or (plant adj allergen))	116	<u>L8</u>
<u>L7</u>	L6 and L5	348	<u>L7</u>
<u>L6</u>	(heterologous or deletion or fusion) same L4	12311	<u>L6</u>
<u>L5</u>	L4 and (allergen)	753	<u>L5</u>
<u>L4</u>	(signal or leader) adj (sequence or peptide)	43160	<u>L4</u>
<u>L3</u>	L2 and ((signal or leader) adj (sequence or peptide))	2	<u>L3</u>
<u>L2</u>	L1 and (allergen)	14	<u>L2</u>
<u>L1</u>	Raz-Eyal.in.	28	<u>L1</u>

## pET-11a-d Vectors

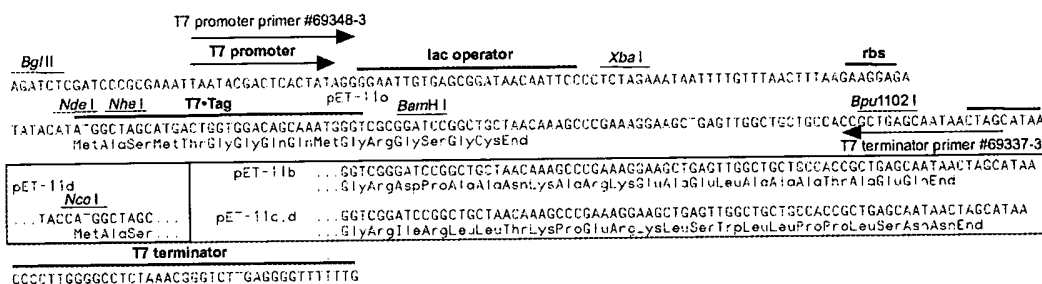
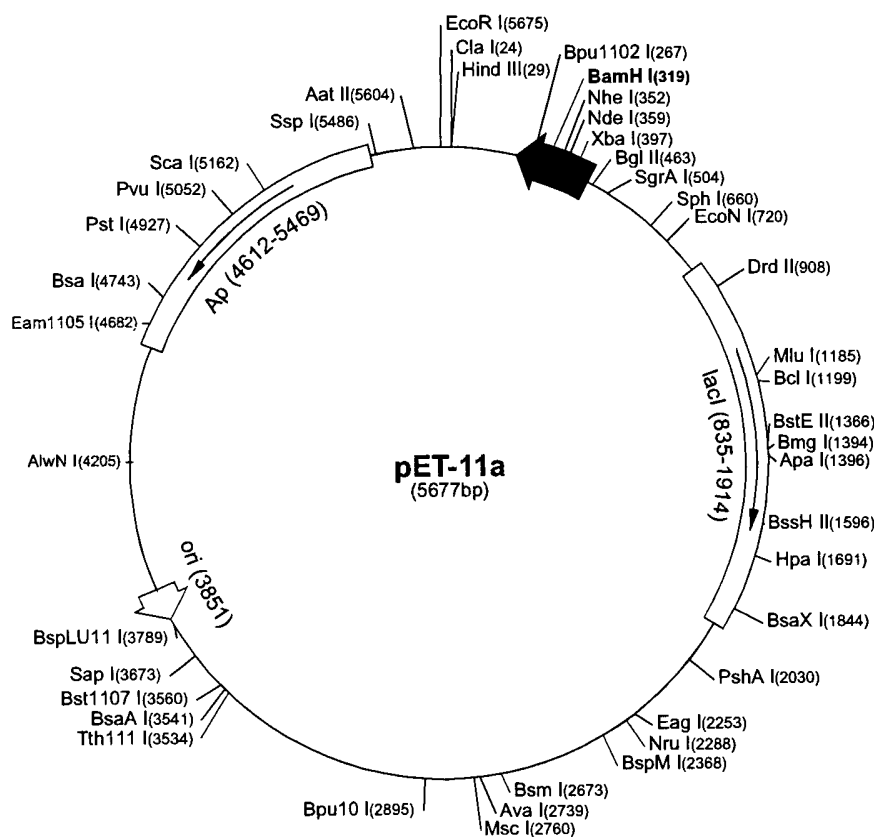
	Cat. No.
pET-11a DNA	69436-3
pET-11b DNA	69437-3
pET-11c DNA	69438-3
pET-11d DNA	69439-3

The pET-11a-d vectors carry an N-terminal T7•Tag<sup>®</sup> sequence and *Bam*H I cloning site. These vectors are the precursors to many pET family vectors; the pET-21a-d(+) series corresponds to pET-11a-d but incorporates several additional features. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below.

### pET-11a sequence landmarks

T7 promoter	432-448
T7 transcription start	431
T7•Tag coding sequence	328-360
T7 terminator	213-259
<i>lacI</i> coding sequence	835-1914
pBR322 origin	3851
<i>bla</i> coding sequence	4612-5469

The maps for pET-11b, pET-11c and pET-11d are the same as pET-11a (shown) with the following exceptions: pET-11b is a 5676bp plasmid; subtract 1bp from each site beyond *Bam*H I at 319. pET-11c is a 5675bp plasmid; subtract 2bp from each site beyond *Bam*H I at 319. pET-11d is a 5674bp plasmid; the *Bam*H I site is in the same reading frame as in pET-11c. An *Nco* I site is substituted for the *Nde* I site with a net 1bp deletion at position 359 of pET-11c. As a result, *Nco* I cuts pET-11d at 355. For the rest of the sites, subtract 3bp from each site beyond position 360 in pET-11a. *Nde* I does not cut pET-11d.



### pET-11a-d cloning/expression region

# pET-11a Restriction Sites

TB042 12/98

Enzyme	# Sites	Locations
AatI	1	5604
AccI	1	3559
AccII	7	952 1680 2011 3298 3439 3741 4981
AcII	89	
AflII	2	1185 3789
AluI	24	
AlwI	16	
Alw21I	8	685 1169 2492 2783 3607 4107 5268 5353
Alw44I	4	1165 3603 4103 5349
AlwNI	1	4205
Apal	1	1396
ApaBI	2	869 2366
ApoI	2	1460 5675
AvaI	1	2739
Avall	9	1737 2113 2201 2450 2753 2795 3074 4820 5042
BamHI	1	319
BanI	12	
BanII	3	569 583 1396
BbsI	5	1331 1670 2044 2907 5660
BbvI	27	
BccI	15	
Bce83I	7	208 1999 2169 3880 4178 4419 5287
BceII	5	704 1045 1672 2481 4291
BcgI	10	315 349 1477 1511 2011 2045 3366 3400 5187 5221
BclI	1	1199
BfaI	7	257 353 398 2803 4284 4537 4872
BglI	3	2249 2483 4802
BglII	1	463
BmgI	1	1394
BpmI	6	1023 1512 2146 2700 3316 4752
Bpu10I	1	2895
Bpu1102I	1	267
BsaI	1	4743
BsaAI	1	3541
BsaBI	3	462 468 2986
BsaHI	8	508 529 643 1142 1825 2520 5219 5601
BsaJI	10	115 129 244 622 628 1820 2481 2683 2761 3949
BsaWI	7	189 1504 2067 2978 3995 4142 4973
BsaXI	1	1844
BsbI	2	3505 5225
BscGI	13	
BsgI	3	1036 1236 2949
Bsil	3	3962 5346 5653
BsiEI	6	1970 2256 3705 4129 5052 5201
BslI	22	
BsmI	1	2673
BsmAI	7	882 1287 1413 1800 3430 4743 5519
BsmBI	2	1800 3430
BsmFI	4	646 2187 2412 3060
BsoFI	50	
Bsp24I	14	
Bsp1286I	11	
BspEI	2	189 2978
BspGI	3	2373 2450 3315
BspLU11I	1	3789
BspMI	1	2368
BsrI	26	
BsrBI	3	418 3722 5523
BsrDI	4	1232 1598 4743 4917

Enzyme	# Sites	Locations
BsrFI	8	160 485 504 871 2083 2243 2597 4762
BssHII	1	1596
Bst1107I	1	3560
BstEII	1	1366
BstXI	3	987 1116 1239
BstYI	11	
CacBI	42	
CjeI	28	
CjePI	26	
Clal	1	24
CviJI	95	
CviRI	26	
DdeI	11	
DpnI	29	
DraI	3	4548 4567 5259
DrdI	2	3482 3897
DrdII	1	908
DsaI	2	622 2761
EaeI	6	493 625 1859 2253 2758 5070
EagI	1	2253
Eam1105I	1	4682
EatI	3	803 3673 5477
EcII	5	962 2709 3863 4009 4837
Eco47III	3	590 2091 3043
Eco57I	2	4337 5349
EcoNI	1	720
EcoO109I	5	240 618 2753 2795 5658
EcoRI	1	5675
EcoRII	10	129 908 1223 1763 1820 2372 2755 3815 3936 3949
EcoRV	2	187 1635
FauI	18	
FokI	14	
FspI	3	2672 2770 4904
GdiII	5	493 625 1859 2253 5070
HaeI	8	913 2234 2306 2363 2760 3804 3815 4267
HaeII	13	
HaeIII	27	
HgaI	15	
HgiEII	2	783 4375
HhaI	44	
Hin4I	5	16 1084 2455 4681 4755
HincII	2	1691 5223
HindIII	1	29
HinII	14	
HpaI	1	1691
HphI	17	
MaeII	12	
MaeIII	18	
MbolI	15	
MluI	1	1185
MmeI	2	4004 4188
MnlI	33	
MscI	1	2760
MseI	24	
MsII	10	1237 1525 1555 2345 2776 2971 3362 4934 5093 5452
MspI	35	
MspA1I	10	271 1215 1785 1878 2455 3380 3499 4131 4376 5317
MwoI	45	
NarI	5	508 529 643 1825 2520
NciI	14	
NdeI	1	359
NgoAIV	4	495 2083 2243 2597
NheI	1	352
NlaIII	31	
NlaIV	28	
NruI	1	2288

Enzyme	# Sites	Locations
NspI	4	660 3134 3426 3793
Pfi1108I	2	2072 4700
PfIMI	3	767 2635 2684
PleI	7	446 734 821 1617 3683 4168 4671
PshAI	1	2030
Psp5II	2	2753 2795
Psp1406I	5	847 2215 3114 4908 5281
PstI	1	4927
PvuI	1	5052
PvuII	3	1785 1878 3380
RcaI	4	583 4509 5517 5622
RsaI	4	165 1332 3595 5162
SapI	1	3673
Sau96I	21	
Sau3AI	29	
ScaI	1	5162
ScrFI	24	
SfaNI	24	
SfcI	5	138 431 4054 4245 4923
SgrAI	1	504
SphI	1	660
Sspl	1	5486
StyI	2	244 2683
TaqI	12	
TaqII	8	1093 1311 1984 3691 5030 5215 5368 5385
TfiI	7	1864 2166 2320 2618 2839 3343 3764
ThaI	40	
TseI	27	
Tsp45I	9	124 1366 2194 2461 3228 3441 3536 4938 5149
Tsp509I	16	
Tth111I	1	3534
Tth111II	7	1024 1717 3250 4379 4386 4418 5674
UbaJI	23	
VspI	4	446 1870 1929 4854
XbaI	1	397
XcmI	3	1041 1557 1575
XmnI	2	3347 5281

Enzymes that do not cut pET-11a:

AflII	AgeI	AscI	AvrII	BaeI
BseRI	BsrGI	Bsu36I	DraIII	FseI
KpnI	MunI	NcoI	NotI	NsiI
NspV	PacI	PmeI	PmlI	RleAI
RsrII	SacI	SacII	Sall	SexAI
SfiI	SglI	SmaI	SnaBI	SpeI
SrfI	Sse8387I	StuI	SunI	Swal
XhoI				



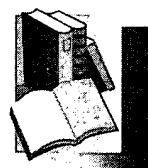
## pMAL™-p2 DNA: Location of Sites

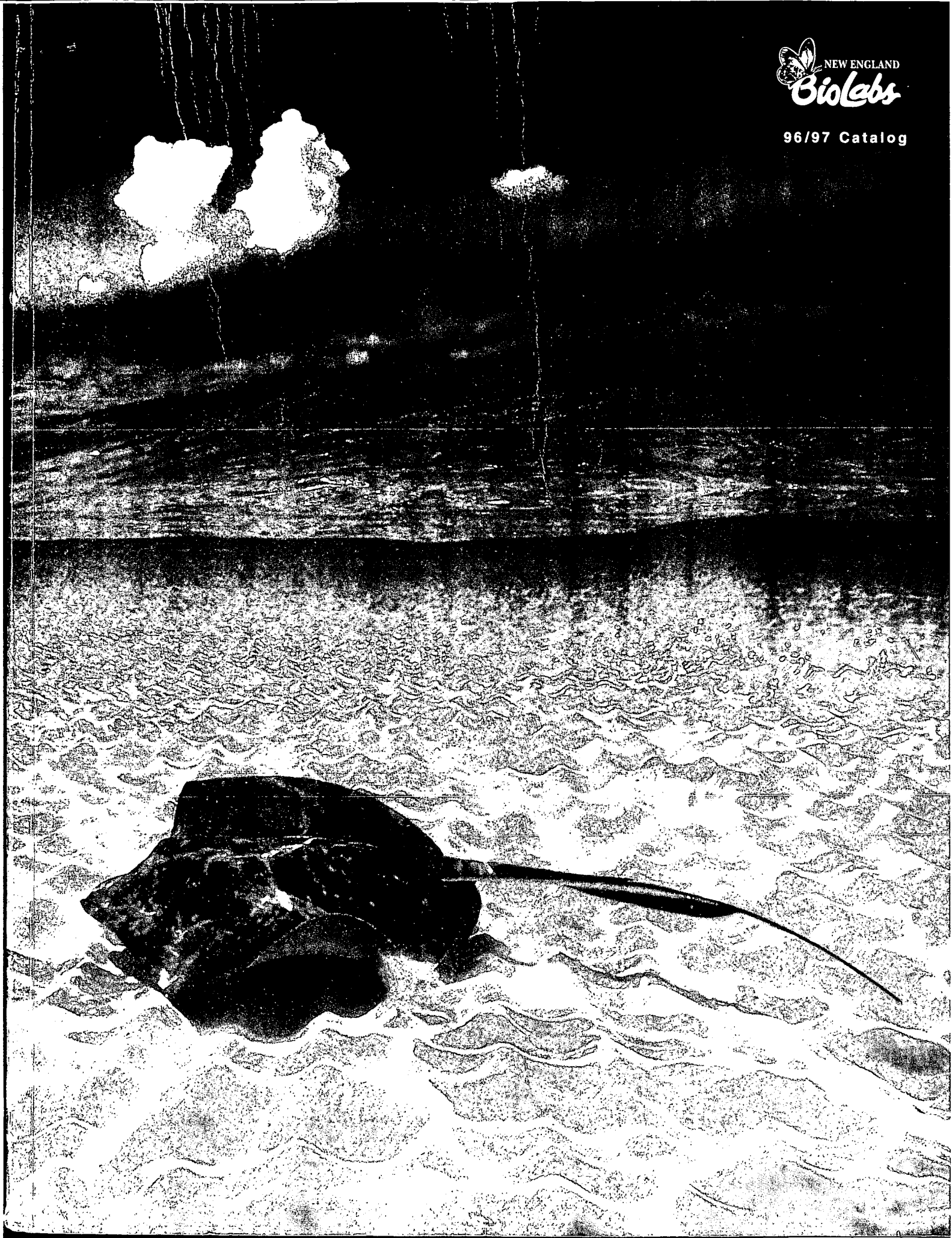
Enzyme	#	Locations		
<i>Ahd I/Eam1105 I</i>	1	4275		
<i>Apa I/Bsp120 I</i>	1	638		
<i>BamH I</i>	1	2776		
<i>Bgl II</i>	1	1962		
<i>Blp I</i>	1	2388		
<i>BsiW I</i>	1	1893		
<i>Bsm I</i>	1	2217		
<i>BspLU11 I</i>	1	5663		
<i>Bst1107 I</i>	1	5892		
<i>BstE II</i>	1	612		
<i>Dra III</i>	1	4672		
<i>Ecl136 II/Sac I</i>	1	2709		
<i>EcoR I</i>	1	2770		
<i>EcoR V</i>	1	879		
<i>Hind III</i>	1	2802		
<i>Hpa I</i>	1	935		
<i>Kas I/Nar I</i>	1	1070		
<i>Mlu I</i>	1	431		
<i>Msc I</i>	1	6694		
<i>Nae I/NgoM I</i>	1	4778		
<i>Nco I</i>	1	2564		
<i>Nde I</i>	1	5841		
<i>PflM I</i>	1	7		
<i>Pst I</i>	1	2794		
<i>Sal I</i>	1	2788		
<i>Sap I</i>	1	5785		
<i>Sca I</i>	1	3797		
<i>Sse8387 I</i>	1	2793		
<i>Sty I</i>	1	2564		
<i>Swa I</i>	1	4449		
<i>Tth1111 I</i>	1	5916		
<i>Xba I</i>	1	2782		
<i>Xmn I</i>	1	2759		
<i>Acc I</i>	2	2788	5892	
<i>Afl III</i>	2	431	5663	
<i>AlwN I</i>	2	2243	5249	
<i>Ava I/BsoB I</i>	2	2746	4566	
<i>Bcl I</i>	2	445	2645	
<i>Bgl I</i>	2	2951	4156	
<i>BsaB I</i>	2	2274	6466	
<i>BsmB I</i>	2	1040	6014	
<i>BsmF I</i>	2	3079	6378	
<i>BspE I</i>	2	1722	6474	
<i>BspH I</i>	2	3440	4943	
<i>BssH II</i>	2	842	2039	
<i>Dra I</i>	2	3700	4450	
<i>Eco47 III</i>	2	2511	6411	
<i>EcoO109 I</i>	2	6657	6699	
<i>PpuM I</i>	2	6657	6699	
<i>Ban II</i>	3	638	2709	4748
<i>Bbs I</i>	3	570	909	6538
<i>Bcg I</i>	3	734	3753	6067
<i>BsaA I</i>	3	2549	4675	5911
<i>Bsa I</i>	3	1660	3140	4208
<i>BsrF I</i>	3	117	4195	4778
<i>BssS I</i>	3	1496	3611	5490
<i>Drd I</i>	3	4626	5555	5968
<i>Dsa I</i>	3	2290	2564	6691
<i>Fsp I</i>	3	2945	4055	6684
<i>Nsp I</i>	3	5663	6028	6322
<i>Pvu I</i>	3	1925	2925	3908
<i>Xcm I</i>	3	280	796	814

Enzyme	#	Locations				
<i>Ase</i> I	4	1115	1174	1412	4104	
<i>Bpm</i> I	4	284	737	4190	6155	
<i>BspM</i> I	4	1294	2054	2395	2792	
<i>BsrD</i> I	4	479	837	4039	4221	
<i>BstX</i> I	4	226	355	478	1981	
<i>Eco57</i> I	4	1667	2515	3593	5136	
<i>Pvu</i> II	4	1029	1122	2895	6072	
<i>Ssp</i> I	4	1387	3473	4446	4467	
<i>Tli</i> I	4	1110	5689	6108	6614	
<i>Ban</i> I	5	351	1070	2503	4327	4714
<i>BsaH</i> I	5	387	1070	1257	3318	3739
<i>BsrB</i> I	5	1446	3270	3436	4819	5732
<i>Eae</i> I	5	1105	2077	2813	3887	6694
<i>Slc</i> I	5	2794	4034	4896	5207	5398
<i>ApaL</i> I	6	411	1241	1544	3608	5349
		5847				
<i>Bla</i> I	6	2783	4087	4828	4917	5170
		6651				
<i>Bsg</i> I	6	297	497	1234	1292	6101
		6488				
<i>Hinc</i> II	6	935	1405	2191	2360	2788
		3736				
<i>Psp1406</i> I	6	92	3281	3677	4050	4461
		6339				
<i>BsaW</i> I	7	750	1696	1722	3984	5310
		5457	6474			
<i>Ear</i> I	7	54	1735	1993	2530	2911
		3486	5785			
<i>BsiE</i> I	8	1797	1925	2661	2925	3759
		3908	5326	5750		
<i>Csp6 I/Rsa</i> I	8	577	1894	2067	2130	2341
		2636	3798	5858		
<i>Ple</i> I	8	59	855	2786	4281	4604
		4626	5293	5764		
<i>Alw26</i> I	9	123	528	654	1041	1661
		3141	3444	4209	6015	
<i>Apo</i> I	9	706	1681	1741	2770	3051
		4479	4490	6248	6717	
<i>Ava</i> II	9	983	1516	2300	3078	3916
		4138	6379	6658	6700	
<i>Tsp45</i> I	9	613	1708	2834	3808	4019
		4851	5914	6009	6222	
<i>BsiHKA</i> I	10	411	1241	1499	1544	2709
		3608	3693	5349	5847	6673
<i>Msl</i> I	10	479	767	797	2212	3505
		3864	4023	6088	6481	6676
<i>BstY</i> I	11	1962	1997	2544	2776	3633
		3650	4913	4925	5011	5022
		6471				
<i>BsaJ</i> I	12	1066	1592	1844	1883	1988
		2290	2564	2745	2847	4396
		5503	6691			
<i>Dde</i> I	12	1003	2389	3102	3192	3248
		3779	4319	4980	5389	5854
		6396	6558			

There are no restriction recognition sites for the following enzymes:

*Aat II, Acc65 I/Kpn I, Afl II, Age I, Asc I, Avr II, BseR I, BspD I/Cla I, BsrG I, BstB I, Bsu36 I, Eag I, EcoN I, Fse I, Mfe I, Nhe I, Not I, Nru I, Nsi I/Ppu10 I, Pac I, PaeR7 I, Xho I, Pme I, Pml I, PshA I, Rsr II, Sac II, SexA I, Sfi I, Sgf I, SgrA I, Sma I/Xma I, SnaB I, Spe I, Sph I, Srf I, Stu I*





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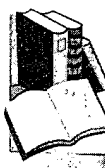
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## pGEX Vectors (GST Gene fusion)

### ORDERING INFORMATION

Product	Quantity	Code Number
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#### Glutathione S-transferase Gene Fusion Vectors\*

pGEX-1λT <i>Eco</i> R I/BAP	5 µg	27-4805-01
pGEX-2T	25 µg	27-4801-01
pGEX-2TK	25 µg	27-4587-01
pGEX-3X	25 µg	27-4803-01
pGEX-4T-1	25 µg	27-4580-01
pGEX-4T-2	25 µg	27-4581-01
pGEX-4T-3	25 µg	27-4583-01
pGEX-5X-1	25 µg	27-4584-01
pGEX-5X-2	25 µg	27-4585-01
pGEX-5X-3	25 µg	27-4586-01
pGEX-6P-1	25 µg	27-4597-01
pGEX-6P-2	25 µg	27-4598-01
pGEX-6P-3	25 µg	27-4599-01

**\* All vectors include *E. coli* BL21 cells.**

**All of the GST gene fusion vectors offer:**

- **A *tac* promoter for chemically inducible, high-level expression.**
- **An internal *lac I<sup>q</sup>* gene for use in any *E. coli* host.**
- **Very mild elution conditions for release of proteins from the affinity matrix, thus**



minimizing effects on antigenicity and function.

- PreScission™, thrombin, or factor Xa protease recognition sites for cleaving the desired protein from the fusion product.

Thirteen pGEX vectors are available (see figure 1). Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors including  $\lambda$  ExCell Cloning Vector (27-5013-01; see  $\lambda$  ExCell Not I/EcoR I/CIP and  $\lambda$  ExCell EcoR I/CIP for more details) and  $\lambda$  ZAP. pGEX-6P-1, pGEX-6P-2, and pGEX-6P-3 encode the recognition sequence for site-specific cleavage by PreScission™ Protease; see PreScission™ Protease between the GST domain and the multiple cloning site. pGEX-4T-1, pGEX-4T-2, and pGEX-4T-3 are derived from pGEX-2T and contain a thrombin recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX-5X-3 are derivatives of pGEX-3X and possess a factor Xa recognition site.

pGEX-2TK is uniquely designed to allow the detection of expressed proteins by directly labeling the fusion products *in vitro* (1). This vector contains the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase obtained from muscle. The protein kinase site is located between the GST domain and the MCS. Expressed proteins can be directly labelled using protein kinase with [ $\gamma$ -<sup>32</sup>P]ATP and readily detected using standard radiometric or autoradiographic techniques. pGEX-2TK is a derivative of pGEX-2T; its fusion proteins can be cleaved with thrombin.

Cleavage of pGEX-6P GST fusion proteins occurs

between the Gln and Gly residues of the rec sequence Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro temperature (5°C) digestion minimizes the degradation of the protein of interest. Because PreScission™ Protease has been engineered GST-tag, it can also be removed from the cleavage mixture simultaneously with the GST portion of the fusion protein. The pGEX-6P Expression Vectors permit convenient site-specific cleavage and simultaneous purification on Glutathione Sepharose™. The pGEX-6P series provides a translational reading frames linked between the GST coding region and the multiple cloning site.

Collectively, the pGEX vectors provide all the translational reading frames beginning with the EcoR I restriction site. pGEX-1λT, pGEX-6P-1, pGEX-4T-1, and pGEX-5X-1 can directly accept and express cDNA inserts isolated from λ gt11 libraries.

Click on "ASCII" to download an unformatted sequence for use by a sequence analysis program. Click on "PDF" to download a formatted sequence and restriction site table. If you prefer access to the sequence in GenBank, refer to the right-hand column for the GenBank accession number:

			GenBank
Vector	Unformatted	Formatted	Accession
pGEX-4T-1, 27-4580-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-4T-2, 27-4581-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-4T-3, 27-4583-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-5X-1, 27-4584-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-5X-2, 27-4585-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-5X-3, 27-4586-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-2TK, 27-4587-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-2T, 27-4801-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-3X, 27-4803-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1385
pGEX-1 lambda T, 27-4805-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U1384
pGEX-6P-1, 27-4597-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U7887
pGEX-6P-2, 27-4598-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U7887
pGEX-6P-3, 27-4599-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U7887

### Properties of pGEX Vectors • Induction: tac | inducible with 1-5 mM IPTG.

- **Expression:** Proteins are expressed as fusion proteins with the 26 kDa glutathione S-transferase (GST). The GST gene contains an ATG and ribosome-binding site, and is under the control of the tac promoter. A translation terminator is provided in each reading frame. The resulting fusion protein may be purified using the GST Purification Module (27-4580-02; see GST Purification Modules.)
- **Enzymatic cleavage with PreScission™ Protease:** pGEX-6P-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with PreScission™ Protease. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed simultaneously with the GST portion of the fusion protein.
- **Enzymatic cleavage with thrombin:** pGEX-2T, -2TK, -3X allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with thrombin.

**lambda T, pGEX-2T, pGEX-2TK, pGEX-4T-3 allow for removal of the GST carrier p from the fusion protein by enzymatic cleavage with thrombin.**

- **Enzymatic cleavage with factor Xa: pGEX-2T, pGEX-5X-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with factor Xa.**
- **Direct labelling *in vitro*: pGEX-2TK allow for direct labelling of fusion proteins *in vitro* with <sup>32</sup>P using the catalytic subunit of cAMP-dependent protein kinase.**
- **Host(s): *E. coli*. The plasmid provides lacZ as a repressor.**
- **Selectable marker(s): Plasmid confers resistance to 100 µg/ml ampicillin.**
- **Amplification: Recommended.**

**• pGEX-2T Control Regions:**

\* **Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 240-257; Start codon (ATG) for GST: 258; Coding region for GST: 258-935; Thrombin cleavage: 918-935**

\* **MCS: 930-945**

\* **Beta-lactamase gene region: Promoter: -1309-1314; -35: 1286-1291; Start codon (ATG): 1356; Stop codon (TAA): 2214**

\* ***lacIq* gene region: Start codon (GTG): 320-335; Stop codon (TGA): 4377**

\* **Plasmid replication region: Site of replication initiation: 2974; Region necessary for replication: 2281-2977**

\* **Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1020-998**

**• pGEX-2TK Control Regions:**

\* **Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 240-257; Start codon (ATG) for GST: 258; Coding region for GST: 258-935; Thrombin cleavage: 918-935**

**217-237; Ribosome binding site for GST: 24 codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935;**

**\* Coding for kinase recognition site: 936-9**

**\* MCS: 951-966**

**\* Beta-lactamase gene region: Promoter: -1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235**

**\* *lacIq* gene region: Start codon (GTG): 33 codon (TGA): 4398**

**\* Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998**

**Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019**

**• pGEX-3X Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* O<sub>2</sub>: 217-237; Ribosome binding site for GST: 24 codon (ATG) for GST: 258; Coding region for Xa cleavage: 921-932**

**\* MCS: 934-949**

**\* Beta-lactamase gene region: Promoter: -1313-1318; -35: 1290-1295; Start codon (ATG): 1360; Stop codon (TAA): 2218**

**\* *lacIq* gene region: Start codon (GTG): 33 codon (TGA): 4381**

**\* Plasmid replication region: Site of replication initiation: 2978; Region necessary for replication: 2285-2981**

**\* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1024-1002**

**• pGEX-1 Lambda T Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* O<sub>2</sub>: 217-237; Ribosome binding site for GST: 24**

**codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935**

**\* MCS: 930-944**

**\* Beta-lactamase gene region: Promoter: -1308-1313; -35: 1285-1290; Start codon (ATG): 1355; Stop codon (TAA): 2213**

**\* *lacIq* gene region: Start codon (GTG): 32 codon (TGA): 4376**

**\* Plasmid replication region: Site of replication initiation: 2973; Region necessary for replication: 2280-2976**

**\* Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1019-997**

**• pGEX-4T-1 Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lacO*: 217-237; Ribosome binding site for GST: 24 codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935**

**\* MCS: 930-966**

**\* Beta-lactamase gene region: Promoter: -1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235**

**\* *lacIq* gene region: Start codon (GTG): 33 codon (TGA): 4398**

**\* Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998**

**\* Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1041-1019**

**• pGEX-4T-2 Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lacO*: 217-237; Ribosome binding site for GST: 24 codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935**

**\* MCS: 930-967**

**\* Beta-lactamase gene region: Promoter: -1331-1336; -35: 1308-1313; Start codon (ATG): 1378; Stop codon (TAA): 2236**

**\* *lacIq* gene region: Start codon (GTG): 33 codon (TGA): 4399**

**\* Plasmid replication region: Site of replication initiation: 2996; Region necessary for replication: 2303-2999**

**\* Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1042-1020**

**• pGEX-4T-3 Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244-257; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935**

**\* MCS: 930-965**

**\* Beta-lactamase gene region: Promoter: -1329-1334; -35: 1306-1311; Start codon (ATG): 1376; Stop codon (TAA): 2234**

**\* *lacIq* gene region: Start codon (GTG): 33 codon (TGA): 4397**

**\* Plasmid replication region: Site of replication initiation: 2994; Region necessary for replication: 2301-2997**

**\* Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1040-1018**

**• pGEX-5X-1 Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244-257; Start codon (ATG) for GST: 258; Coding region for Xa cleavage: 921-932**

**\* MCS: 934-969**

**\* Beta-lactamase gene region: Promoter: -**

**1333-1338; -35: 1310-1315; Start codon (ATG): 1380; Stop codon (TAA): 2238**

**\* *lacIq* gene region: Start codon (GTG): 333; Stop codon (TGA): 4401**

**\* Plasmid replication region: Site of replication initiation: 2998; Region necessary for replication: 2305-3001**

**\* Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1044-1022**

**. pGEX-5X-2 Control Regions:**

**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Xa cleavage: 921-932**

**\* MCS: 934-970**

**\* Beta-lactamase gene region: Promoter: -1334-1339; -35: 1311-1316; Start codon (ATG): 1381; Stop codon (TAA): 2239**

**\* *lacIq* gene region: Start codon (GTG): 333; Stop codon (TGA): 4402**

**\* Plasmid replication region: Site of replication initiation: 2999; Region necessary for replication: 2306-3002**

**\* Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1045-1023**

**. pGEX-5X-3 Control Regions:**

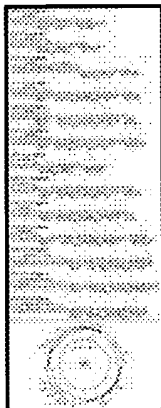
**\* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Xa cleavage: 921-932**

**\* MCS: 934-971**

**\* Beta-lactamase gene region: Promoter: -1335-1340; -35: 1312-1317; Start codon (ATG): 1382; Stop codon (TAA): 2240**



- \* ***lacIq* gene region: Start codon (GTG): 33 codon (TGA): 4403**
- \* **Plasmid replication region: Site of replication initiation: 3000; Region necessary for replication: 2307-3003**
- \* **Sequencing primers: 5' pGEX Sequencing primer binds nucleotides 869-891; 3' pGEX Sequencing primer binds nucleotides 1046-1024**



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***Map of the glutathione S-transferase fusion showing the reading frames and main features. Even though stop codons in all three frames depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.***

## References

1. Kaelin, W.G. et al., *Cell* 70, 351 (1992).

<b>Related Products</b>	<b>Code Number</b>
<u>GST Purification Modules</u>	
<u>GST Detection Module (50 detections)</u>	27-4590-01
<u>GSTrap™ FF Columns</u>	
<u>GSTPrep FF 16/10</u>	17-5234-01
<u>Glutathione Sepharose™ 4B Lab Packs and Columns</u>	
<u>PreScission Protease</u>	27-0843-01
<u>Anti-GST Antibody (50 detections)</u>	27-4577-01
<u>GST Vector Primers for Sequencing</u>	
<u>E. coli BL21</u>	27-1542-01

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